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CO_2 Production as a Measure of Decay Activity in Wood Blocks

CO_2-produktion som ett mått på rötaktivitet i träklossar

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Introduction

Basically, a decay process in wood can be described as an aerobic breakdown of wood under the development of CO_2 and H_2O. Less intense processes, not investigated in this paper, can also take place under anaerobic conditions.

Weight-loss tests are the most common method for measuring the decay (HEARTLEY 1958). These tests are time consuming and are fraught with various practical difficulties.

The purpose of this investigation was to study the CO_2 output of decaying wood. A simple method which made very small demands on laboratory equipment was used. The method is compared with weight-loss tests.

Materials and methods

Ordinary 100 ml Erlenmeyer flasks with wide necks and rubber plugs were used for the *CO_2 measurements*. 5 ml of 0.05 N NaOH was put into each flask. The test blocks (mean size 2 × 1.5 × 5 cm) were fastened to the plugs with needles. The blocks were dipped into the flasks for 5 hours and then removed, and 0.15 ml of saturated $BaCl_2$ was added to precipitate as $BaCO_3$ the Na_2CO_3 obtained, whereupon a titration was made with 0.05 N HCl to determine the remaining NaOH. Indicator: phenolphthalein. Some dry sterile blocks were always included, giving a test error which had to be subtracted.

From the consumed NaOH the amount of CO_2 was calculated as mg CO_2 per g dry weight and 24 hours. Dry weight equals the weight after 48 hours at + 101°C. In a few orientating experiments the CO_2 is given as mg CO_2 per mg N of the wood (total amount of N according to Kjeldahl analysis performed at the State Control Institute for Agricultural Research).

Experiments with *weight-loss* in sterile blocks of wood: Ocularly homogeneous blocks were weighed after 48 hours at + 101°C, heated again for a further 3 hours to make them sterile, then moistened in sterile distilled water to 25—30 % (moisture content of the dry weight), and finally put in Petri dishes containing cultures of the fungus in question for 15 days in one-litre glass containers with water in the bottom to ensure a high relative air humidity (12—14 blocks to a dish). After the incubation time the blocks were transferred to sterile glass jars with water in the bottom and small containers of NaOH to remove the CO_2 from respiration (5 blocks in each jar, air volume 0.9 litre). This treatment was performed under sterile conditions.

During the test time it was found necessary to make the air circulate every third day to maintain the oxygen content and to control the NaOH concentration. This was not done in the case of some control jars, in which extreme values of 15 % CO_2 and 2 % O_2 were registered after 60 days. These conditions had a limiting effect on a *Fomes* decay.

After the test time the blocks were dried as described above and the substance loss was calculated as a percentage of the original dry weight.

It will be noticed that, due to a certain transport from the agar (HARTLEY 1958), there is a slight *increase in dry weight* during the incubation time. It is negligible as a weight increase, but not as an increase of nutrient status. There is also a certain *loss of weight* caused by the drying at + 101°C. No mention of this fact has been found in any paper concerning decay in ordinary tests (cf. BURO 1954). If a block of sound wood is weighed after 2 days at + 101°C, then used in a decay experiment and then weighed again after a further 2 days at + 101°C, there is a certain loss (0.1—0.5 %) caused by the heating. In decay tests this loss is included in the loss caused by fungal activity.

In the *weight-loss test concerning natural decay* it is necessary to estimate the dry weight at the start of the test without drying the blocks and killing the fungus. This is achieved by measuring the moisture content in a representative group of control blocks and then referring to this mean moisture quotient for every group of test blocks in the experiment, i.e. the weight at the beginning of the experiment and a relevant moisture quotient give a calculated dry weight which can be compared with a real dry weight at the end of the test.

Logs of spruce (*Picea abies* (L.) Karst.) with naturally established *Fomes annosus* (Fr.) Cke. taken from Garpenberg (near Hedemora) and Tynningö (near Stockholm). The mycelia having numbers L 64 and L 45 in the reference collection of pure cultures of the Department of Forest Botany, Royal College of Forestry. Further, *Peniophora gigantea* (Fr.) Massee K 25, *Stereum sanguinolentum* (A. et S.) Fr. K 29, *Polyporus abietinus* (Dicks.) Fr. L 57, *Lentinus lepideus* Fr. N 14, *Trametes pini* (Thore) Fr. L 50, *Scytalidium album* B. et K. FF 28 and *Trichoderma viride* Fr. F 49 were used.

Experimental

A few words about the CO_2 output of sound spruce wood to be used for control purposes are necessary. The tests on sound wood were carried out 1—2 days after cutting (Sept. 1963). A disc of wood was taken from a 1 metre log just before the tests were started, and test blocks were then cut from the disc and allowed to attain the test temperature (+ 25 °C).

Fig. 1 shows that the N-content and moisture quotient are different in sound sapwood and heartwood. An increase in N-content towards the centre of the heartwood, which is also shown in fig. 1, has previously been found by BECKER 1962 concerning protein. Fig. 2 clearly shows that only the youngest sapwood produces CO_2 vigorously. The heartwood has no CO_2 production.

Both dry weight and N-content were studied as a reference for CO_2 output. It seems appropriate to use dry weight (see figs. 1 and 2) (cf. ŻELAWSKI 1960).

Temperature has a pronounced influence on the CO_2 output, the most intense output being obtained at + 38 °C. Fig. 3 indicates a very probable optimum temperature of 38—40 °C.

Test blocks were cut in the same way from a log naturally decayed by *Fomes annosus* which had been stored outdoors for 4 days after felling (Sept. 1963). Fig. 4 shows that the light firm rot in the heartwood produces CO_2 (+ 25 °C). Generally speaking the moisture content of the heartwood amounting to 31—33 % is too low to promote decay (CARTWRIGHT & FINDLAY 1958). For distinctions between different types of root rot, see BJÖRKMAN ET AL. 1949.

Test blocks from the same log were dipped into sterile water and stored for 2 days in one-litre glass containers with water in the bottom to ensure a higher moisture content. A moisture quotient of 40 % or more increased the CO_2 output by about three times. When leaving a sub-optimal and limiting level, however, the correlation between CO_2 output and moisture is obscure.

Blocks of sound spruce heartwood were prepared in the same way for control purposes, but it was found that a change in moisture content in this test from 34 % to 46 % did not restore CO_2 productivity in sound heartwood.

The N-content was also used in this experiment as a reference for the CO_2 output of decaying wood. When leaving the low original moisture content and intensifying the CO_2 output there is a considerable variation in the separate

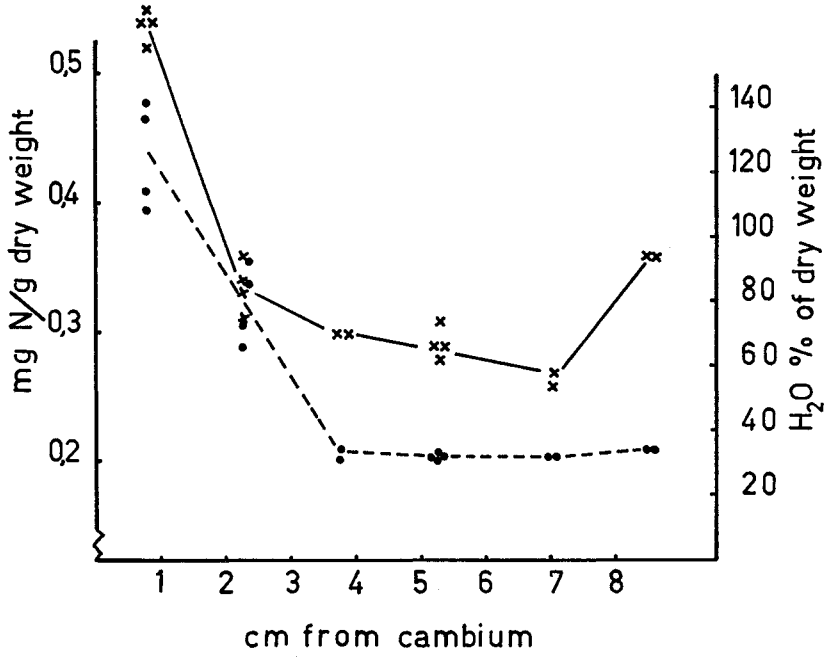

Fig. 1. Total amount of N (Kjeldahl analysis) and moisture quotient (dotted line) in a disc of sound spruce wood, from the cambium to the centre of the trunk.

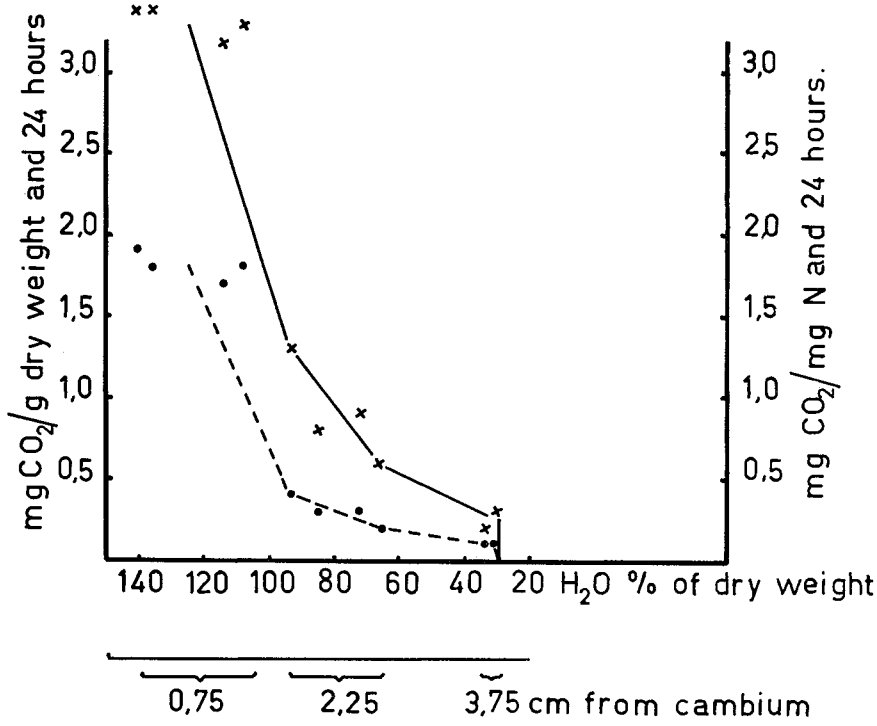

Fig. 2. CO_2 output from wood blocks taken from a disc of sound spruce wood. The relationship between heartwood and sapwood can be seen from the moisture quotient or distance from the cambium. Continuous line = mg CO_2 per mg N, dotted line = mg CO_2 per g dry weight.

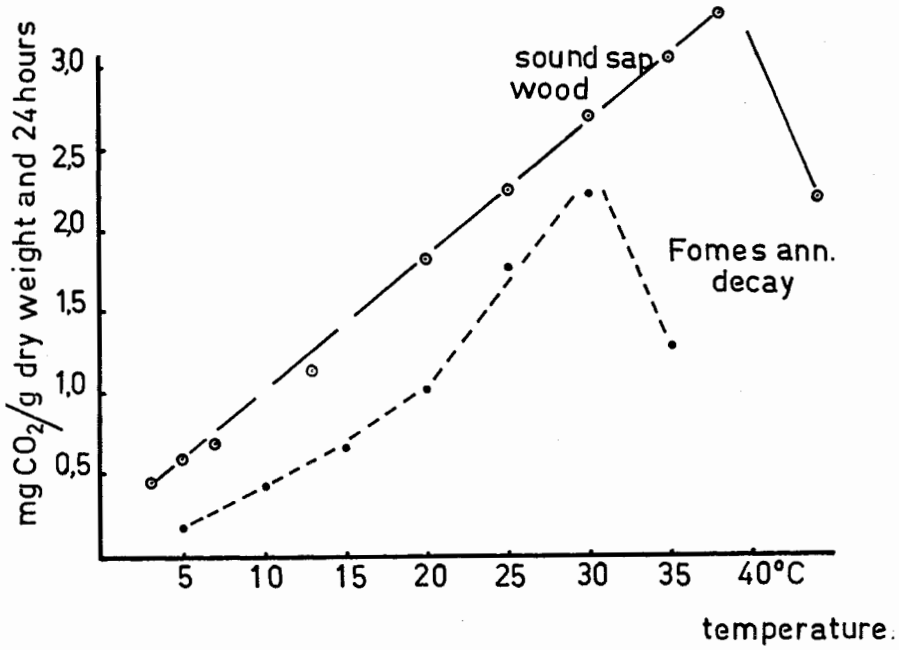

Fig. 3. Temperature curves of CO_2 output from wood blocks — last 1.5 cm of sound sapwood in a disc of sound spruce wood = continuous line — and from naturally established *Fomes annosus* in spruce heartwood = dotted line.

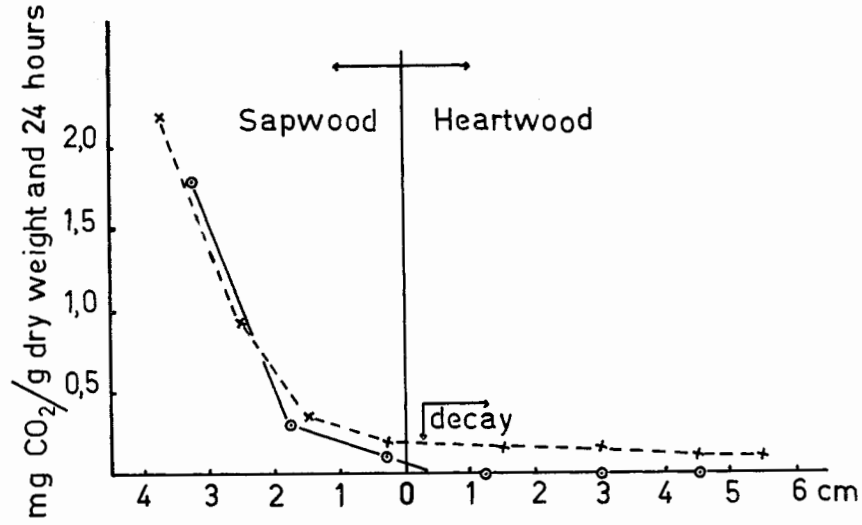

Fig. 4. CO_2 output from wood blocks. Heartwood with naturally established *Fomes annosus* has a low and even CO_2 production (dotted line). Continuous line gives a comparison with wood blocks from a sound trunk.

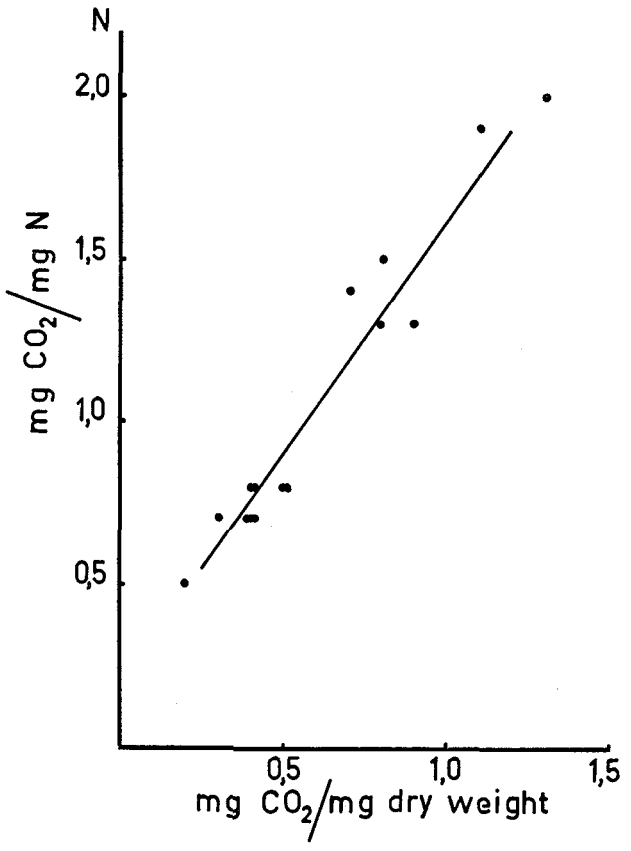

Fig. 5. Comparison between CO_2 values calculated as a function of g dry weight and of mg N.

values, irrespective of whether the calculation is based on the CO_2 output from mg N in the test block or on g dry weight. There is a linear function, however, between those N values and dry weight values (fig. 5). This shows that they are comparable, although there appears to be no advantage in using N values instead of the dry weight.

The following test demonstrates the influence of temperature on the CO_2 output of *Fomes annosus* decay. Test blocks (dark firm rot) were stored for 3 weeks after felling at + 4°C then, as in the previous experiments, given a higher moisture content, and finally placed in a high relative air humidity in the first test temperature (+ 5°C) for 24 hours and measured twice at 24 hour intervals. *The same blocks* were then moved to the next test temperature (+ 10°C) and measured twice after 24 hours at this temperature. This

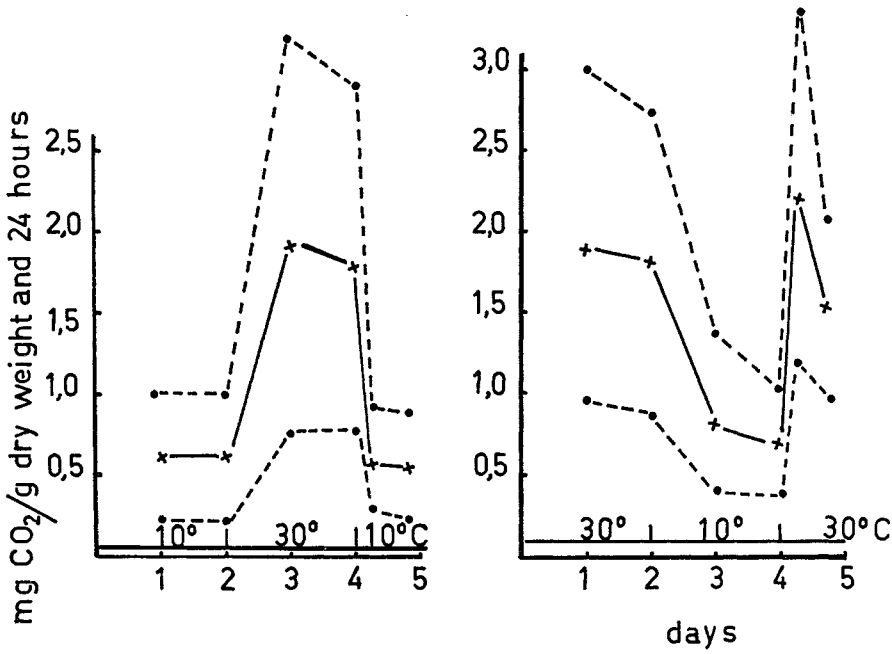

Fig. 6. CO_2 output from wood blocks with naturally established *Fomes annosus* studied at different temperatures. Continuous line = mean values, dotted lines = highest and lowest values in the group of 15 blocks.

Fig. 7. CO_2 output from wood blocks with naturally established *Fomes annosus* studied at different temperatures. Note the tendency for a brief over production of CO_2 after moving from + 10°C to + 30°C. Continuous line = mean values, dotted lines = highest and lowest values in the group of 14 blocks.

procedure was repeated up to a final test temperature of + 35°C. The moisture content was controlled by weighing and was kept at 47—49 %. The reason for repeated measuring at 24 hour intervals is that nothing is known about adaption times to different temperatures.

The most intense CO_2 output was recorded at + 30°C. Fig. 3 indicates a very probable optimum temperature of + 29°C. The radial growth was checked on malt agar with mycelium from the test blocks and the optimum was found to be + 25°C (cf. ROLL-HANSEN, 1940 and CARTWRIGHT & FINDLAY, 1958 with an optimum radial growth + 24°C and 23°C respectively).

The values have a wide variation which is largest at the optimum temperature, but the separate curve for every single test block has the same optimum.

Some further experiments were carried out under different temperature conditions. Two groups of test blocks which had been stored and prepared as above were measured. The first group was measured twice at + 10°C, twice

at + 30°C and again at + 10°C and showed a nice return of the values (fig. 6). The second group was started at + 30°C, moved to + 10°C and then to + 30°C (fig. 7). In both cases there was a quick return to the starting temperature (see fig. 6 and 7) with no adaption period of 24 hours.

It seems possible to trace a stimulating effect of the low temperature period as a short over-production of CO_2 in an ensuing higher temperature. The enzyme production of a mycelium may be remarkably high at low temperatures (GÄUMANN, 1951). After a sudden change to a higher temperature there may be a certain surplus of enzymes or prepared substratum. There are certainly differences between different fungi. COWLING (1961) compares a white rot and a shrink rot and demonstrates a greater depolymerization than is being used up by respiration. SEIFERT (1963) presents similar facts. These facts seem to be reasonably compatible with the possibility of a surplus production of CO_2.

To test the low temperature influence two groups of 15 test blocks were prepared. They were cut from the same log as described in fig. 4. They were given a slight increase in moisture content by being dipped in sterile distilled water and adapted for 3 hours at + 25°C before being measured.

One group was then stored at + 4°C and the other at — 8°C for 18 days. They were then restored to + 25°C and then, after 3 hours at this temperature, the new CO_2 output was measured.

The group stored at + 4°C gave a higher CO_2 production, 0.3 ± 0.02 — 0.4 ± 0.02 mg CO_2/g dry weight, and that stored at — 8°C a lower CO_2 production 0.2 ± 0.01 — 0.1 ± 0.01 than the original values. These indications cannot be generalized. It is only a suggestion that measuring CO_2 can be used.

All these tests with CO_2 output from decay have been tried where weight loss tests are impracticable. The advantage of the method is perhaps lost if it is used in protracted tests involving risks of mould infection and other errors.

A single test was performed with a group of test blocks of the same origin as those used in the temperature experiments (figs. 3, 6 and 7). They were given a high initial moisture content (95 %) and were then allowed to dry slowly for 45 days to 20 %.

In this case (fig. 8) mean values and mean errors are shown for CO_2 values at each 10 % interval of moisture content (95—85 %, 85—75 %, etc.). No details are shown in the mean figure curve which illustrates a general trend only. Changes of moisture content between 40—80 % appear to be of limited importance.

At the end of the test time mycelia of *Ceratocystis* sp., *Pullularia* sp. and *Penicillium* sp. were found in some blocks, partly as secondary infections. The CO_2 values from some of these blocks have two separate maxima. In

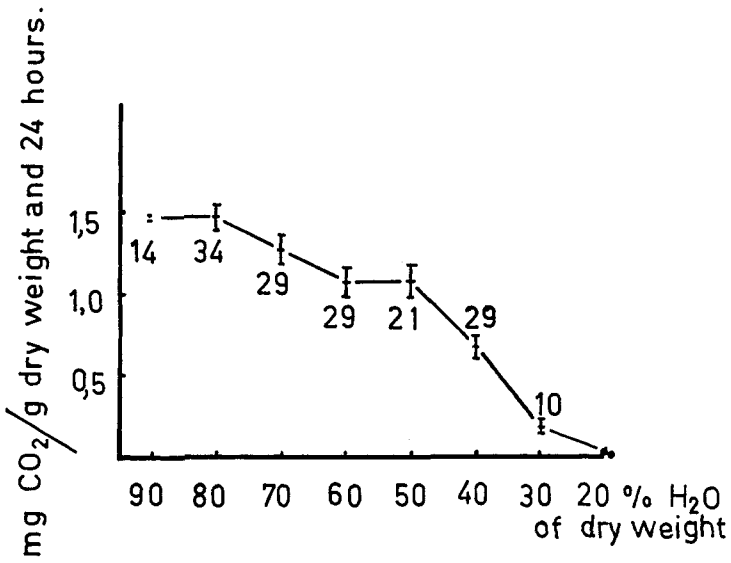

Fig. 8. CO_2 output from wood blocks with naturally established *Fomes annosus* during a slow drying process at + 25 °C. Mean values and standard errors (⊤|⊥) are shown within each 10 % interval of the moisture quotient. The figures in the diagram = number of observations.

the mean value curve these become mere irregularities of no statistical significance.

It is necessary to check this CO_2 method against weight loss tests. Some preliminary tests were carried out to ascertain whether it is possible to compare the information obtained from this CO_2 method with ordinary weight loss tests. The effects of secondary infections and preservatives in relation to CO_2 measurements will also be commented upon.

The loss of weight was followed in groups of test blocks of sterile spruce wood incubated on agar cultures of *Fomes annosus* (mycelium taken from the same log as in figs. 4 and 5), *Peniophora gigantea* and *Stereum sanguinolentum* at + 25 °C (fig. 9). The CO_2 output was measured at every weight loss reading. During the limited test time the loss of weight seems to have a constant rate of about 2—3 % of dry weight a month with corresponding CO_2 values of approximately 1.5 mg CO_2 per g dry weight and 24 hours.

In another test the effect of naturally established decay of *Fomes annosus* was studied (wood blocks of the same origin as in figs. 4 and 5). It is difficult to make an adequate estimate of the dry weight at the beginning of a test

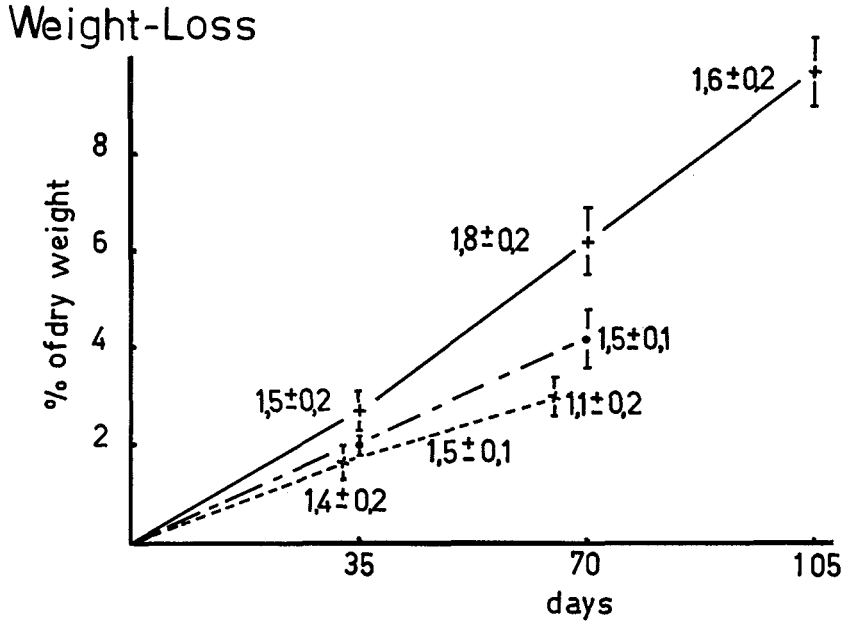

Fig. 9. Weight loss and CO_2 output from sterile single cultures in spruce wood blocks at + 25°C. The figures in the diagram = mg CO_2 pr g dry weight and 24 hours at each reading of weight loss.
+———+ *Peniophora*
●—·—·—● *Stereum*
×·····× *Fomes*

(Standard errors of weight loss are indicated by ⊤+⊥).

with natural decay, which makes the standard errors rather great. But the weight loss is of the same magnitude as in the foregoing test concerning sterile wood blocks and the CO_2 values are about the same (fig. 10).

The following test is an attempt to compare two decay processes of different intensity by cultivating *Fomes annosus* at + 10°C and + 20°C. + 20°C gives both a greater weight loss and a higher CO_2 production.

In the next test two fungi (*Trametes pini* and *Lentinus lepideus*) which cause more rapid decomposition are studied in sterile wood blocks. *Trametes pini* is the same type of decay fungus as those studied in the above experiments and have a certain ability to attack both cellulose and lignin (CARTWRIGHT & FINDLAY 58). The CO_2 values of *Trametes* (fig. 11) are much higher and in reasonable correlation with the less intense *Fomes*, *Peniophora* and *Stereum* (figs. 9, 10 and 11). *Lentinus lepideus*, which is a specialist at splitting cellulose, gives a greater weight loss, but on the other hand the CO_2 values are much lower. Different fungi obviously have different correlations between

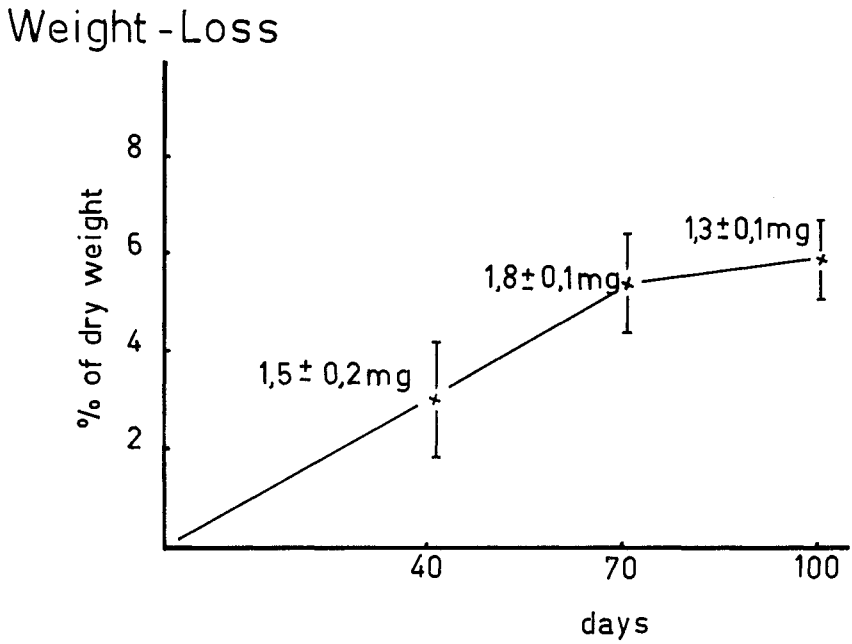

Fig. 10. Weight loss and CO_2 output from naturally established *Fomes annosus* in spruce wood blocks at + 25°C. The figures in the diagram = mg CO_2 per g dry weight and 24 hours at each reading of weight loss.

(Standard errors of weight loss are indicated by ⊤|⊥).

weight loss and CO_2 production. The highest CO_2 production occurs in the beginning of the test and is followed by successively lower values in spite of an equal weight loss. These may be a disturbing influence from the inoculation on agar cultures, and furthermore little is known about the initial breakdown of different wood substances.

There is sometimes a great difference between separate values and mean values. In one case concerning naturally established *Fomes annosus* in spruce wood (fig. 10) two wood blocks were contaminated with *Trichoderma viride* and one with *Chaetomium sp.* The blocks with *Trichoderma* gave only 0.1 and 0.3 mg CO_2 per g dry weight and 24 hours, compared with 1.5 and 1.8 mg CO_2 respectively as an average for uncontaminated blocks. The wood blocks with *Chaetomium sp.* gave 0.6 mg CO_2 compared with 1.3 mg CO_2 which suggests that the effect of *Chaetomium* on *Fomes* is less intense than that of *Trichoderma.*

Another fungus, *Scytalidium album,* not earlier described in Sweden, was

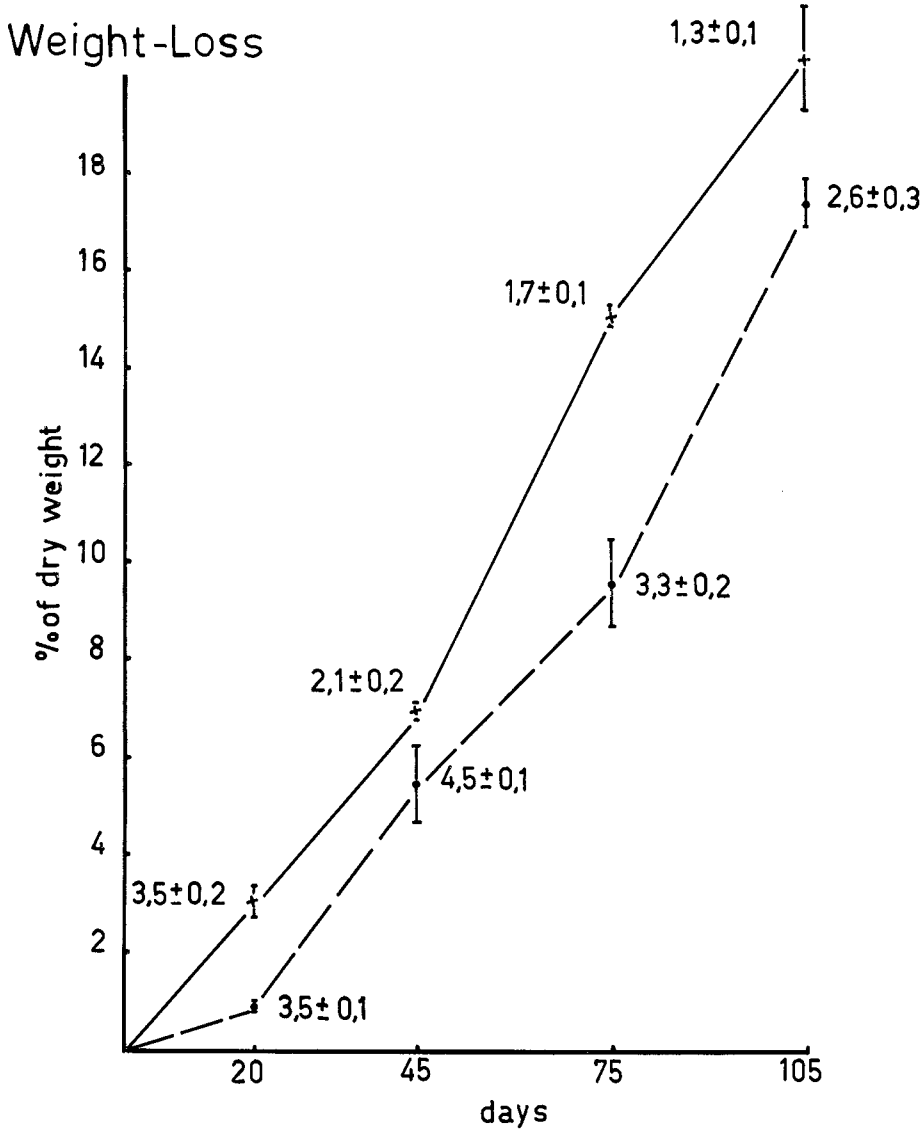

Fig. 11. Weight loss and CO_2 output from sterile single cultures in spruce wood blocks at + 25°C.
+———+ *Lentinus lepideus*
· — — — · *Trametes pini*
The figures in the diagram = mg CO_2 per g dry weight and 24 hours at each reading of weight loss.

(Standard errors of weight loss are indicated by ⊢+⊣).

found in trunks with *Fomes* decay and proved to be very antagonistic to *Fomes*. (KLINGSTRÖM & BEYER 1965).

In the final experiment CO_2 measurements were carried out before and after treatment with two potent chemicals to see the effect on a naturally established decay of *Fomes annosus*. A quick method using CO_2 could be complementary to ordinary studies of preservatives. Four groups of wood blocks (the same origin as in fig. 10) were studied three times at + 25°C:

1) to find a starting value prior to treatment
2) 15 hours after treatment
3) 50 hours after treatment

The first group was an untreated control group, the second was treated with distilled water, the third group was treated in the same way with 0.5 % Na-pentachlorphenol in water, and the last group with 50 ppm cycloheximide in water. (The phenol compound is a very common preservative—CARTWRIGHT & FINDLAY 1958. Cf. KUHLMAN & HENDRIX 1962 who states that also related compounds e. g. pentachloronitrobenzene, can be used in very low concentrations in selective media for *Fomes*. Cycloheximide has been studied in relation to *Fomes* and found to have a strong fungistatic effect —GUNDERSEN, 1962).

Treatment with these chemicals has a pronounced effect on the CO_2 production (table 1). Pentachlorphenol causes a reduction of the CO_2 values; cycloheximide gives a pronounced increase of the CO_2 output. A partial stimulation of CO_2 production by antifungal agents has previously been recorded by PAGANO ET AL. (1961—62). The purpose of this paper is not to discuss these values but to suggest as a principle that measuring CO_2 can be used in studying preservatives.

Table 1. Effect on CO_2 production of decaying wood *(Fomes annosus)* by treatment with water, Na-pentachlorphenol, and cycloheximide.

Mg CO_2/g dry weight and 24 h.	Control-Untreated wood	Control-Treated with water	Treated with 0,5 % Na-pentachlor-phenol	Treated with 50 ppm cyclo-heximide
Before treatment.....	1.3 ± 0,08	0.7 ± 0,09	1.5 ± 0,09	0.6 ± 0,06
15 h after treatment...	1.3 ± 0,13	0.7 ± 0,00	0.9 ± 0,05	1.1 ± 0,07
50 h after treatment...	1.0 ± 0,04	0.7 ± 0,08	1.0 ± 0,04	2.2 ± 0,17
Moisture content in the beginning and at the end of the test.....	53.9—53.0 %	55.9—75.2 %	51.3—87.0 %	49.2—73.8 %

Discussion

The CO_2 output during a very short test period can only reflect the decay intensity during the time in question; a weight loss test on the other hand gives the total loss up till the time of reading.

It is possible to measure the total CO_2 output during a long test instead of weight loss and it would be possible to make a direct comparison of the two methods. There is no point, however, in changing from one slow test method to another incorporating the same difficulties and errors.

There must be a limited correlation between values of weight loss and CO_2 output measured in the way suggested in this paper. Under well controlled conditions in weight loss tests, however, the fungal activity has an equal speed for a considerable time (HARTLEY, 1958). Repeated CO_2 examinations as suggested in this paper combined with registration of weight loss give a rough correlation between the two methods. There may be differences between fungi as there are between woods of different origin, but values of CO_2 output may prove to be a valuable complement to laborious and time-consuming weight loss tests. Further it is possible to study a naturally established decay and to make rough correlations to weight loss from CO_2 values.

The advantage of a method using CO_2 from decay is the ability to measure under conditions where for instance weight loss tests are inadequate. It may for example be of value to study the influence of a sudden change in temperature or moisture conditions, or the influence of various preservatives on a naturally established decay, and it can result in valuable information concerning antibiotic effects between different fungi.

Summary

1. There is a high CO_2 production in the youngest sapwood of newly felled, sound spruce with an optimum temperature of +38—40°C. The CO_2 output diminishes towards the boundary between sapwood and heartwood. The heartwood has no CO_2 production.

2. The CO_2 output of heartwood with naturally established decay of *Fomes annosus* is low and even when measured directly after felling. If blocks with natural decay are moistened slightly and permitted to acclimatise for some days at +25°C in a high relative air humidity a sharp increase in the CO_2 output ensues. The variation in CO_2 output among replicates becomes high irrespective of whether dry weight or N-content is used as a reference for CO_2 output.

3. The CO_2 output of naturally occurring root rot varies to a great extent with changes in temperature; the optimum temperature is +29°C. The storage of wood in low temperatures may affect the intensity of the CO_2 output in later experiments at higher temperatures.

4. The moisture quotient in decaying wood affects the CO_2 output less clearly between wide limits.
Sub-optimum and super-optimum temperatures have a restricting effect however.

5. If the weight loss is measured frequently and found to have a constant rate the CO_2 values also move between close boundaries. This concerns naturally established decay. It is therefore possible to establish CO_2 values quite accurately. In other words one can determine CO_2 values which correspond to a certain intensity of weight loss in the fungus under examination.
In decay tests using sterile wood blocks of known dry weight high CO_2 values can be recorded in the initial phase, which then decrease. This is not reflected by the weight loss, which can have a constant rate in ordinary experiments.

6. CO_2 measurements in decaying wood are highly sensitive for example to changes in temperature and moisture, inoculation with antagonistic fungi or the introduction of preservatives or other poisonous substances. It should be possible to use the method for the study of natural decay processes as it is very difficult to estimate the dry weight at the start of the test without drastically drying the wood, thereby damaging the fungi it is proposed to study.

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Sammanfattning

CO_2-produktion som ett mått på rötaktivitet i träklossar

1. Yttersta splinten har hög CO_2-produktion i nyavverkad frisk granved med temperaturoptimum vid + 38°—40°C. CO_2-avgivningen minskar in mot gränsen mellan splint och kärna. Kärnveden har ingen CO_2-produktion.

2. CO_2-avgivningen från kärnved med naturlig *Fomes annosus*-röta är låg och jämn vid mätning i anslutning till avverkning.

Om klossar med naturlig röta fuktas något och får acklimatiseras några dygn vid + 25°C och hög relativ luftfuktighet, inträder stark ökning av CO_2-avgivningen, och spridningen för CO_2-värdena blir stor. Detta gäller oavsett man använder torrvikt eller totalkväve som referens för CO_2.

3. CO_2-avgivningen från naturligt anlagd rotröta är starkt temperaturberoende med optimum vid + 29°C. Lagring av ved vid låga temperaturer kan påverka CO_2-avgivningens intensitet vid påföljande försök vid högre temperaturer.

4. Fuktkvoten i rötved påverkar CO_2-avgivningen mindre entydigt inom vida gränser. Sub- och supraoptimala värden verkar dock hämmande.

5. Om substansförlust mätes vid upprepade tillfällen och befinnes ha en konstant hastighet pendlar också CO_2-värdena inom snäva gränser. Detta gäller naturligt anlagd rotröta. Man kan alltså fastställa CO_2-värden som motsvarar en viss intensitet av substansförlusten för den undersökta svampen. Olika svamparter och olika röttyper kan inte utan vidare jämföras. Vid rötförsök med sterila torrviktsbestämda träklossar kan initialskedet giva höga CO_2-värden, som sedan sjunker. Detta återspeglas vanligen ej av substansförlusten, som kan vara jämnt kontinuerlig.

6. CO_2-mätningar på röta i ved ger känsliga utslag för t. ex. förändringar av temperatur och fuktighet, införande av antagonistiska svampar eller tillförande av impregneringsmedel eller andra giftiga ämnen. Metoden bör kunna användas för studier av naturligt anlagda rötprocesser, då det är mycket besvärligt att uppskatta en torrvikt vid start av försök utan att drastiskt torka veden och därmed skada de svampar man vill studera.